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RESEARCH COMMENTARIES

IMMUNOLOGY:

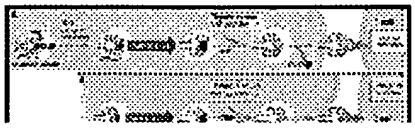
Proteases, Processing, and Thymic Selection

Peter Cresswell

Foreign proteins internalized by cells are degraded into peptides. These are displayed on the cell surface bound to molecules called MHC class II (major histocompatibility complex class II). It is only in this context that the $CD4^+$ T cells of the immune system can recognize these foreign peptides. The peptide binding site of MHC class II is blocked during assembly and intracellular transport by a transmembrane glycoprotein called the invariant chain (1). MHC class II-invariant chain complexes are delivered into the endocytic system, where the invariant chain is degraded by endosomal and lysosomal proteases, collectively known as cathepsins. A fragment of the invariant chain, CLIP (class II-associated invariant chain peptide), remains as a place holder in the binding site until its dissociation is induced by interaction of the class II molecules with another class II-like molecule (H-2M in mice and HLA-DM in humans). The unoccupied binding site is then available for peptide fragments from degraded foreign proteins. The class II-peptide complexes so generated are then transported to the cell surface.

Which cathepsins degrade the invariant chain? This question can be answered with the use of specific chemical inhibitors of individual cathepsins (2-5) or, recently, "knockout" mice, which lack expression of certain cathepsins. One of these, which is missing cathepsin L, is reported by Nakagawa *et al.* on page 450 of this issue (6). The surprising result is that the key cathepsin or cathepsins that degrade the invariant chain and thereby generate functional class II molecules are different in different tissues.

There are two main places where the generation of MHC class II-peptide complexes is important—in the thymus and in peripheral tissues. In the thymus, developing T cells encounter MHC molecules that display peptides derived from self proteins (7). In the currently favored model, $CD4^+$ T cells that recognize particular class II-self peptide complexes with moderate affinity mature and escape into the periphery (positive selection). Those recognizing class II-self peptide complexes with high affinity, which might cause autoimmune disease if allowed to escape, are deleted (negative selection). $CD4^+$ T cells with receptors below a certain threshold of affinity "die by neglect" and also fail to exit the thymus. Similar processes govern the development of $CD8^+$ T cells that recognize MHC class I-peptide complexes. Positive selection is carried out by class II-positive cortical epithelial cells in the thymus, whereas negative selection is performed by medullary bone marrow-derived dendritic cells and macrophages (see the figure).



Thymic display table. MHC class II is processed in thymic cortical epithelial cells and bone marrow-derived antigen-presenting cells to display peptides. The cortical epithelial cells (**A**) mediate positive selection of $CD4^+$ T cells in the thymus and use cathepsin L for the late stages of invariant chain degradation. Bone marrow-derived antigen-presenting cells (**B**) in the thymic medulla mediate negative selection, and peripheral bone marrow-derived antigen-presenting cells present foreign peptides associated with MHC class II molecules to antigen-specific $CD4^+$ T cells. They appear to use cathepsin S for the late stages of invariant chain degradation.

In peripheral tissues the key MHC class II-positive cell types are B cells, macrophages, and dendritic cells—collectively known as antigen-presenting cells. As well as constitutively expressing class II-self peptide complexes, these cells can express class II molecules associated with peptides derived from foreign, pathogen-derived proteins internalized during the course of an infection. These complexes are recognized in the lymph nodes or spleen by specific, mature $CD4^+$ T cells that multiply and release a variety of cytokines that amplify and modulate the overall immune response.

Cathepsin L-deficient mice exhibit a defect in the MHC class II processing pathway in thymic cortical epithelial cells. Specifically, invariant chain degradation in these cells does not proceed normally, and partial proteolytic fragments derived from it accumulate in association with class II molecules. These fragments include the CLIP region, and similar products have been previously characterized in class II-positive cells treated in vitro with inhibitors of the cysteine protease family of cathepsins (2-5). Presumably because of defective invariant chain degradation and consequent expression of a reduced repertoire of class II-peptide complexes on the cortical epithelial cells, positive selection is seriously impaired. The number of $CD4^+$ T cells in the thymus and periphery is reduced by 60 to 80%, whereas the number of $CD8^+$ cells increases to maintain the overall number of T cells. In contrast, in the bone marrow-derived medullary population responsible for negative selection, and in the peripheral class II-positive cells mediating the presentation of foreign peptides to mature $CD4^+$ T cells, the MHC class II processing pathway appears normal. No proteolytic fragments of invariant chain accumulate in spleen cells of the cathepsin L-deficient mice, and their splenocytes, dendritic cells, and macrophages are perfectly capable of generating class II-peptide complexes from a variety of cellular and internalized soluble protein antigens, stimulating specific $CD4^+$ T cells as efficiently as wild-type cells.

Cathepsin L is thus a critical protease for invariant chain degradation in thymic epithelium. But is there another enzyme that fulfills the same role in peripheral antigen-presenting cells? Evidence obtained by treating antigen-presenting cells in vitro with specific inhibitors suggests that cysteine proteases in general—and cathepsin S in particular—may be important in the periphery. Cathepsin S inhibition induces the accumulation of a partially proteolyzed fragment of the invariant chain in association with class II molecules and inhibits peptide loading (4, 5). Nakagawa *et al.* (6) show that in thymus of normal mice, active cathepsin S is undetectable in cortical epithelial cells but present in thymus-derived dendritic cells and peripheral antigen-presenting cells, whereas active cathepsin L has a reciprocal distribution. Thus, the terminal stages of invariant chain degradation in thymic epithelium and in bone marrow-derived antigen-presenting cells appear to be mediated by different cathepsins.

Why should the critical invariant chain processing enzyme in thymic cortical epithelial cells differ from that in bone marrow-derived antigen-presenting cells? The range of self peptides presented to T cells undergoing negative selection in the thymus should be the same as those presented in the periphery, otherwise the potential for autoimmune recognition is high. Because the cathepsins, in addition to degrading the invariant chain, are responsible for generating the class II-associated peptides, it may be important for the proteases of the negatively selecting thymic medullary cells to be similar to those in the peripheral antigen-presenting cells. Such a restriction need not be imposed on the cortical epithelial cells mediating positive selection, where the only requirement is that a broad T cell receptor repertoire be generated. Thus, the biology of the system can be said to allow the difference in cathepsin L distribution, but the reason for it remains unclear. A further complexity is that an alternatively spliced form of the invariant chain, generally expressed together with the major form and called p41, incorporates a specific cathepsin L inhibitory domain (8, 9). Does this domain regulate the activity of cathepsin L in the thymus or periphery? As is often the case, this important observation raises more questions than it answers.

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The author is in the Section of Immunobiology, Howard Hughes Medical Institute Research Laboratories, Yale University School of Medicine, 310 Cedar Street, New Haven, CT 06510, USA. E-mail: peter.cresswell@yale.edu